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ORIGINAL ARTICLE

The use of polymerase chain reaction in the diagnosis of invasive meningococcal disease☆

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Abstract
Introduction, objectives and aims: Invasive meningococcal disease (IMD) remains a serious public health problem. Although culture is the gold standard, previous antibiotic therapy reduces its sensibility. The aim of this study is the epidemiological analysis of IMD in our area, to assess the usefulness of polymerase chain reaction (PCR) to increase its diagnostic accuracy, and to show the association of antibiotic administration with the negative result of the culture.
Patients and methods: A retrospective study was conducted on all children younger than 16 years with microbiologically (positive culture and/or PCR) confirmed IMD, admitted to our hospital between 2004 and 2012.
Results: Seventy-five patients were included, of whom 52% had sepsis, 30.7% meningitis, and 17.3% with both. PCR showed positive results in all samples, whereas a positive result was seen in 50.7% of the cultures. Previously administered antibiotic was documented in 40 patients (53.3%), and 40% of them were confirmed by PCR only.
Conclusions: PCR was the only test providing evidence for IMD diagnosis and serogroup determination in almost 39% of cases.

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PALABRAS CLAVE
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La reacción en cadena de la polimerasa en el diagnóstico de la enfermedad meningocócica invasiva

Resumen
Introducción objetivos: La enfermedad meningocócica invasiva (EMI) constituye un grave problema de salud pública. A pesar de que el cultivo es la técnica de referencia para su diagnóstico, la administración previa de antibiótico altera su sensibilidad. Los objetivos de este estudio son el análisis epidemiológico de la EMI en nuestro medio, evaluar la utilidad de la reacción en cadena de la polimerasa (PCR) para incrementar el diagnóstico de confirmación de la EMI y valorar la asociación de la administración de antibiótico con el resultado negativo del cultivo.
Pacientes y métodos: Estudio retrospectivo de los pacientes menores de 16 años diagnosticados de EMI mediante cultivo, PCR o ambos, que ingresaron en nuestro centro en el periodo 2004–2012.
Resultados: Se incluyó a 75 pacientes, de los cuales el 52% presentó sepsis, el 30,7% meningitis y el 17,3% sepsis con meningitis. La PCR fue positiva en todas las muestras de sangre y líquido cefalorraquídeo analizadas, mientras que el cultivo tuvo una positividad muy inferior (50,7%). Recibieron antibiótico antes de la extracción de las muestras 40 pacientes (53,3%) y el 40% de ellos fueron confirmados por la PCR.
Conclusiones: Gracias a la PCR se obtuvo un diagnóstico de confirmación de EMI en el 38,7% de los casos y del serogrupo, hecho relevante para la vigilancia epidemiológica y el estudio de la efectividad vacunal.

Introduction

Invasive meningococcal disease (IMD) constitutes a serious public health problem worldwide, with a high impact on society and a high rate of morbidity and mortality in all age groups. Despite advances in our knowledge of the disease and its diagnosis and treatment, and the implementation of new preventive strategies, IMD is endemic in many countries, even developed ones, with attack rates of between 1 and 5 cases per 100,000 inhabitants.[1]

In Spain, according to data from the national system of epidemiological surveillance (Sistema Nacional de Vigilancia Epidemiológica [SNVE]) from 2004 to 2011, the overall incidence rate for IMD was of 0.92–1.67 cases per 100,000 inhabitants.[2–4] The highest incidence in children occurs in those younger than 1 year, with a rate of 13 cases per 100,000 in the 2009–2010 season.[3] Serogroups B and C caused over 90% of the IMD cases in Spain.[2,3,5] The introduction of the conjugate meningococcal C vaccine in 2001 has reduced the incidence of IMD caused by this serotype by up to 88%,[6] but it is still a serious disease due to its sequelae (11–19%)[2,3] and mortality rate (5–14%).[1–3]

The diagnosis of IMD is confirmed by ascertaining the presence of *Neisseria meningitidis* (*N. meningitidis*) in the blood or in the cerebrospinal fluid (CSF), or in both, either by culture or by detection of bacterial deoxyribonucleic acid (DNA),[5,7] or the two methods. Culture continues to be the gold standard for microbiological diagnosis, and it also allows for antimicrobial sensitivity and strain identification testing of the isolate. Still, it does have limitations, chief among which is its low diagnostic yield when the samples are taken from patients who have received previous antibiotic treatment.[5,8–11]

Even if the culture is performed, there is a sizable percentage of reported IMD cases without a microbiological diagnosis, and this limits the analysis of its epidemiological evolution and the impact of vaccination.

The introduction of DNA detection techniques that use polymerase chain reaction (PCR) represents an advance in confirmatory diagnosis, and various studies and clinical practise guidelines recommend routine PCR testing.[5,8,12–14]

The aim of this study was to make an epidemiological analysis of IMD in our country, assess the usefulness of PCR in the diagnosis of IMD, and study the correlation of negative cultures in confirmed cases with previous antibiotic therapy.

Patients and methods

We performed a retrospective study of the medical records of patients younger than 16 years with a clinical diagnosis of IMD confirmed by culture, PCR, or both, admitted to the Hospital Universitari Vall d'Hebron between January 2004 and December 2012. All the cases were selected based on culture and PCR results from the microbiology department.

We collected data on demographic, clinical, and laboratory variables, and also documented whether the patient had received antibiotic therapy prior to sample collection.

Samples of blood, CSF or both were collected for culture and PCR (both techniques were performed on a single sample), and were processed and analysed in the molecular diagnosis, blood culture, and general culture laboratories of the microbiology department. PCR testing was done on samples larger than 100 μL. Nucleic acids were extracted from the samples with the *EasyMag*® automated platform (bioMèrieux, Marcy-l'Étoile, France) and later amplified by multiplex PCR for *N. meningitidis* (ctrA